

## § 113.308

## 9 CFR Ch. I (1–1–00 Edition)

from each dog and tested for neutralizing antibody to canine distemper virus in the same manner used to determine susceptibility.

(iii) If the two controls have not remained seronegative at 1:2, the test is inconclusive and may be repeated.

(iv) If at least 6 of the 8 vaccinates in a valid test do not develop titers of at least 1:50 based upon final serum dilution, the Master Seed Virus is unsatisfactory, except as provided in paragraph (b)(4)(v) of this section.

(v) If the results of a valid serum neutralization test are unsatisfactory, the vaccinates and the controls may be challenged as provided in paragraph (b)(3) of this section. A Master Seed is satisfactory if all vaccinates remain free of clinical signs of canine distemper, while the two controls die with clinical signs of canine distemper. If the two controls do not die with clinical signs of canine distemper, the test is inconclusive and may be repeated: *Provided*, That, if any of the vaccinates show such signs, the Master Seed Virus is unsatisfactory.

(5) An Outline of Production change shall be made before authorization for use of a new lot of Master Seed Virus shall be granted by the Animal and Plant Health Inspection Service.

(c) *Test requirements for release.* Except for § 113.300(a)(3)(ii), each serial and subserial shall meet the requirements prescribed in § 113.300 and in this paragraph. Final container samples of completed product shall be tested. Any serial or subserial found unsatisfactory by a prescribed test shall not be released.

(1) The test for pathogens prescribed in § 113.37 shall be conducted on each serial or one subserial of avian origin vaccine.

(2) *Virus titer requirements.* Final container samples of completed product shall be tested for virus titer using the titration method used in paragraph (b)(2) of this section. To be eligible for release, each serial and subserial shall have a virus titer sufficiently greater than the titer of vaccine virus used in the immunogenicity test prescribed in paragraph (b) of this section to assure that when tested at any time within the expiration period, each serial and subserial shall have a virus titer of  $10^{0.7}$

greater than that used in such immunogenicity test but not less than  $10^{2.5}$  TCID<sub>50</sub> per dose.

[60 FR 14362, Mar. 17, 1995]

### § 113.308 Encephalomyelitis Vaccine, Venezuelan.

Encephalomyelitis Vaccine, Venezuelan, shall be prepared from virus-bearing cell culture fluids. Only Master Seed which has been established as pure, safe, and immunogenic shall be used for preparing seeds for vaccine production. All serials of vaccine shall be prepared from the first through the fifth passage from the Master Seed.

(a) The Master Seed shall meet the applicable general requirements prescribed in § 113.300 except (b), and the requirements prescribed in this section.

(b) Each lot of Master Seed shall be tested for immunogenicity. The selected virus dose from the lot of Master Seed shall be established as follows:

(1) Tests conducted by the Department have established that horses having Venezuelan equine encephalomyelitis antibody titers of 1:20 by the hemagglutination-inhibition (HI) method or 1:40 by the serum neutralization (SN) method were immune to challenge with virulent virus. The immunogenicity test is based on the demonstration of a serological response of at least that magnitude following vaccination of serologically negative horses.

(2) At least 22 horses (20 vaccinates and 2 controls), susceptible to Venezuelan equine encephalomyelitis, shall be used as test animals. Blood samples shall be taken from each horse and the serums individually tested for neutralizing antibody. Horses shall be considered susceptible if there is no neutralization at a 1:2 final serum dilution in a constant virus-varying serum neutralization test using 60 to 300 TCID<sub>50</sub> of Venezuelan equine encephalomyelitis virus.

(3) A geometric mean titer of the vaccine produced from the highest passage of the Master Seed shall be established using a method acceptable to Veterinary Services before the immunogenicity test is conducted. The 20 horses used as vaccinates shall be injected with a predetermined quantity

of vaccine virus by the method to be recommended on the label. To confirm the dosage administered, five replicate virus titrations shall be conducted on a sample of the vaccine virus dilution used.

(4) Twenty-one to twenty-eight days postvaccination, blood samples shall be drawn from all test animals. For a valid test, the controls shall remain seronegative at 1:2 final serum dilution. In a valid test, if at least 19 of 20 vaccinates do not have antibody titers of at least 1:20 in a hemagglutination-inhibition test or at least 1:40 in a serum neutralization test, the Master Seed is unsatisfactory.

(5) The Master Seed shall be retested for immunogenicity in 3 years unless use of the lot is discontinued. Only five vaccinates and two controls need to be used in the retest: *Provided*, That five of five vaccinates and the two controls shall meet the criteria in paragraph (b)(4) of this section.

(6) An Outline of Production change shall be made before authority for use of a new lot of Master Seed shall be granted by Animal and Plant Health Inspection Service.

(c) *Test requirements for release.* Each serial and subserial shall meet the applicable general requirements prescribed in §113.300 and special requirements in this paragraph. Any serial or subserial found unsatisfactory by a prescribed test shall not be released.

(1) *Safety test.* The mouse safety test prescribed in §113.33(b) shall be conducted.

(2) *Virus titer requirements.* Final container samples of completed product shall be tested for virus titer using the method in paragraph (b)(3) of this section. To be eligible for release, each serial and subserial shall have a virus titer sufficiently greater than the titer of the vaccine used in the immunogenicity test prescribed in paragraph (b) of this section to assure that, when tested at any time within the expiration period, each serial and subserial shall have a virus titer of  $10^{0.7}$  greater than that used in the immunogenicity test, but not less than  $10^{2.5}$  TCID<sub>50</sub> per dose.

[50 FR 23797, June 6, 1985. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66786, Dec. 26, 1991]

#### § 113.309 Bovine Parainfluenza<sub>3</sub> Vaccine.

Bovine Parainfluenza<sub>3</sub> Vaccine shall be produced from virus-bearing cell culture fluids. Only Master Seed Virus which has been established as pure, safe, and immunogenic shall be used for preparing the production seed virus for vaccine production. All serials of vaccine shall be prepared from the first through the tenth passage from the Master Seed Virus.

(a) The Master Seed Virus shall meet the applicable general requirements prescribed in §113.300.

(b) Each lot of Master Seed Virus shall meet the special requirements prescribed in this section.

(c) Each lot of Master Seed Virus used for vaccine production shall be tested for immunogenicity. The selected virus dose from the lot of Master Seed Virus shall be established as follows:

(1) Twenty-five bovine parainfluenza, susceptible calves shall be used as test animals (20 vaccinates and five controls). Blood samples shall be drawn from these animals and individual serums tested. Also, nasal specimens shall be collected for virus isolation attempts. The calves shall be considered susceptible if:

(i) The results are negative at a 1:2 final serum dilution in a varying serum constant virus neutralization test with less than 500 TCID<sub>50</sub> of bovine parainfluenza<sub>3</sub> virus; and

(ii) Shall be negative to bovine parainfluenza<sub>3</sub> virus isolation attempts from the nasal specimens on the day of injection.

(2) A geometric mean titer of the dried vaccine produced from the highest passage of the Master Seed Virus shall be established before the immunogenicity test is conducted. The 20 calves to be used as vaccinates shall be injected with a predetermined quantity of vaccine virus and the remaining five calves held as uninjected controls. To confirm the dosage calculation, five replicate virus titrations shall be conducted on a sample of the vaccine virus dilution used.

(3) The vaccinates and controls shall be examined for clinical signs of respiratory disease and the body temperature taken and recorded on each of the